

Application No.: 10/796,298
Filing Date: March 9, 2004

AMENDMENTS TO THE SPECIFICATION

Please replace Paragraph [0061] of the specification with the following new paragraph:

[0061] In this embodiment, the method involves the preparation of the filter plate. Either glassfiber membranes or leukocyte filter membranes can be used to capture leukocytes. In order to simplify the assay, multiple-well filterplates are constructed using glassfiber membranes or leukocyte filter membranes to enable the simultaneous processing of multiple blood specimens. Examples of filters for capturing leukocytes are disclosed in U.S. Pat. Nos. 4,925,572 and 4,880,548, the disclosures of which are hereby incorporated by reference. Adsorption of leukocytes on fiber surfaces is generally accepted as the mechanism of leukocyte removal. Since the surface area of a given weight of fibers is inversely proportional to the diameter of the fibers, it is to be expected that finer fibers will have higher capacity and that the quantity as measured by weight of fibers necessary to achieve a desired efficiency will be less if the fibers used are smaller in diameter. A number of commonly used fibers, including polyesters, polyamides, and acrylics, lend themselves to radiation grafting, as they have adequate resistance to degradation by γ -radiation at the levels required for grafting and are of a structure with which available monomers can react. Polybutylene terephthalate (PBT) has been the principal resin used for the development of the products of this invention and is the resin used in the examples. It should be noted, however, that other resins may be found which can be fiberized and collected as mats or webs with fibers as small as 1.5 micrometers or less, and that such products, with their critical wetting surface tensions adjusted as necessary to the optimum range, may be well suited to the fabrication of equally efficient but still smaller leukocyte depletion devices. Similarly, glass fibers, appropriately treated, may be usable to make effective devices. Absorption of CD4 mRNA is up to four times as effective when using PBT-based filters as opposed to glass fiber-based filters. The filter plate is placed in the vacuum box. In another preferred embodiment, multiple filter membranes are layered together to increase the amount of leukocytes captured from whole blood. In one preferred embodiment, the filter plate is placed upon the plate supporter and the sealing gasket. In another preferred embodiment, the filter plate is sealed with a plastic adhesive tape (Bio-Rad 223-9444), and the tape is cut to allow access to a desired number of wells. In

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another preferred embodiment, each well to which a sample will be added is washed with a hypotonic buffer (200 μ L 5 mM Tris, pH 7.4).